

BLACK CUMIN (*NIGELLA SATIVA L*) AFFECTS SOME PARAMETER IN MALE ALBINO RATS

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ABSTRACT

This study aimed to evaluate the probable effect of oil extract of *black* cumin seeds *Nigella sativa L.* on weight body and other body organs (pancrease, spleen, liver and kidney) and some blood parameters (Hb, PCV, WBCs, GPT and GOT). The extract was administrated orally for 30 days at (0.1, 0.2, 0.3, 0.4 ml/100 gm of body weight (b. wt.). day⁻¹) to adult male rats in addition to control group. Body and other organs weight for control and treated rats were measured.

Results of experiment indicate that the extract decreased significantly ($p > 0.05$) the body weight in all groups in contrast with control group, while the weight of pancreas was increased significantly, spleen decreased significantly in 0.2, 0.3, 0.4 mg/gm of b.w. and liver was decreased significantly in 0.2, 0.3, 0.4 mg/gm of b.w, while the kidney was not affected in all treatments. Hematological picture, The result obtained revealed that there were a significant changes ($p < 0.05$) including Hb, PCV% and WBCs. **Histological** Examination of H&E sections of livers of all treatment showed vascular congestion of liver in 0.1 and 0.2 ml/gm b.w, **Spleen tissue** of normal rat administered *N. sativa seed* extract, spleen showed hyperplasia in red pulps in all doses of plant extract. **while the histological examination of kidney and pancreas showed normal** intact histological structure in both control and treated group.

KEYWORDS: *Nigella sativa*, Haematological Parameters, Histology Rats

INTRODUCTION

Herbal medicine is one of the oldest, if not the oldest forms of healing, starting with the origin of human life. The use of herbal medicine is relatively cheap, easily accessed and blends with socio-cultural life of people (1). The use of essential oils as parts of medicinal and aromatic plants for the treatment of diseases is on the increase.

In the present time herbal products are considered as safe alternatives of synthetic drugs that are regarded as unsafe to human and environment. However in the recent past increasing research evidence is getting accumulated, which clearly indicate the positive role of spices and plant extracts for health care. The use of plant drugs is increased in many of the developing countries because modern lives saving drugs are beyond the reach of three quarters of the third world's population. Many of such developing countries spend 40 to 50% of their total wealth on drugs and health care reference. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future.

Nigella sativa (Klonji) is a valuable spice, having distinctive aroma and taste; its seeds have been used in pickles, bread recepies and savoury dishes. *N. sativa* is regarded as a valuable remedy for various ailments, the seeds, oil and extracts have played an important role over the years in ancient Islamic system of herbal medicine. Bukhari reported that Holy Prophet Muhammad (peace be upon him) told" There exists, in the black grains, health care of all the diseases, except

death (2) The dried seeds are used as astringent, bitter, diuretic, emmenagogue; stimulant and anthelmintic. Its decoction is useful in paralysis, jaundice, intermittent fever, dyspepsia, piles and skin diseases (3) and (4) *N. sativa* seeds are reported to contain numerous chemical compounds. Active ingredients are nigellone, beta-sisterol, and thymoquinone. Other compounds include 2-methyl-4-isopropyl-p-quinones, anthraquinones, saponins, glycosides, melanthin, essential oil, fixed oil, tannins, protein, mucilage resins and glucose, etc (2).

The seeds of *Nigella sativa* L. (Ranunculaceae), sometimes known as black seed, black cumin or habatul Barakah have long been used in the Middle East as a traditional medicine for a variety of complaints, headache, cough, flatulence, as a choleric, antispasmodic and uricosuric (5); (6); (7); (8). *Nigella sativa* is presently used in traditional medicine and for culinary preparations in many countries. *N. Sativa* seeds contain 36%–38% fixed oils, proteins, alkaloids, saponin and 0.4%–2.5% essential oil (5). The fixed oil is composed mainly of unsaturated fatty acids including the unusual C20:2 arachidic and eicosadienoic acids (9).

MATERIALS AND METHODS

Plant Material

Seeds extract of *N. sativa* L. was bought from local market.

Animals

Male albino rats aged between (8-10 weeks) from the Animal House of Babylon University were used. Animals were maintained in standard cages and 12-12h light/dark cycle with free access to food and water. The animals were divided into 5 experimental groups of 4 rats per group. Group 1 considered as controlled treatment, while the other groups were daily administered orally with (0.1, 0.2, 0.3, 0.4 ml/100 gm of b.w.) of seeds extract for 30 days. One day after the last treatment all the animals were sacrificed.

Weight of Organ

At the end of the treatment, each rat was sacrificed under ether anaesthesia, the body weight, spleen, kidney, pancreas, and liver for all animals were removed, cleared from adherent tissues, dried and weighed immediately.

Haematological Estimation

Blood was collected from each rat via the left ventricular cardiac puncture into sterilized sample bottle and was allowed to clot at room temperature and then centrifuged at 3000 g for 15 min and the serum separated. The serum samples were stored frozen, and then used for the estimation of haemoglobin (Hb), packed cell volume (PCV), and white blood cell count (WBC) according to the standard techniques described by (10) and (11).

Histological Study

Tissue specimens of liver, kidney and spleen were fixed in 10% formalin saline. Trimming was done on the fixed tissue specimens. Serial alcohol were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 7 microns thickness by microtome. The obtained tissue sections were collected on the glass slides and stained by hematoxylin and eosin stain for histopathological examination by the light microscope (12). (10x).

Assessment of GOT and GPT

Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) were estimated by a UV-Kinetic method based on the reference method of International Federation of ClinicaChemistry (13).

Statistical Analysis

All values were expressed as mean \pm SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) under P values < 0.05 were considered to be statistically significant (14).

RESULTS

Body Weight

As shown in table 1, The oral administration of black cumin extract at all dose levels, the body weights were decreased significantly ($p < 0.05$) in comparison with control treatment.

Table 1: Effect of Different Concentration of *Nigella sativa* L. Seed Extract on Body Weight after Treated for 30 Days

Groups Parameters	Control Group	0.1 ml/gm b.w.	0.2 ml/gm b.w.	0.3 ml/gm b.w.	0.4 ml/gm b.w.
Initial weight	91.5	76.05	82	99.85	104.4
Final weight	190.13	131.751	116.631	148.198	151.867
Changes	+99.8	+55.7	+34.631	+ 59.348	+
Mean of increasing %	a 104.177 \pm 16.742	b 73.236 \pm 1.171	c 52.153 \pm 4.96	c 48.289 \pm 2.946	c 40.491 \pm 3.541
Significant level	*	*	*	*	*

X \pm SE: Mean \pm Standard error, *: Significant effect under ($p < 0.05$), N.S.: Not significant effect, Different symbols mean significant effect.

Organ Weight

The oral administration of black cumin extract at all dose levels changes the organs weight significantly ($p < 0.05$), the pancrease weight increase significantly in 0.3 and 0.4 ml/gm of b.w, the spleen decreased significantly in 0.2, 0.3 and 0.4 ml/gm of b. w, the liver weight decreased significantly in all concentration and kidney weight show no significant changes in comparison with control group (Table 2).

Table 2: Effect of Different Concentration of *Nigella sativa* L. Seed Extract on Weight of Pancrease, Spleen, Liver and Kidney of Adult Male Rats after Treated for 30 Days

Groups Weight(gm)%	Control Group	0.1 ml/gm b.w.	0.2 ml/gm b.w.	0.3 ml/gm b.w.	0.4 ml/gm b.w.	Significant Level
Pancrease	a 0.409	a 0.289	a 0.403	b 0.503	b 0.518	*
spleen	a 0.737	a 0.800	b 0.650	b 0.699	b 0.697	*
Liver	a 4.882	b 3.855	b 4.132	b 4.221	b 4.367	*
kidney	a 0.694	a 0.694	a 0.523	a 0.525	a 0.504	N.S

L.S.D: Less Significant differences under ($p < 0.05$) for pancreas = 0.066, kidney = 0.046.

Different symbols mean significant effect, *: Significant effect under ($p < 0.05$).

Hematological picture: The result obtained revealed that there were a significant changes ($p < 0.05$) including Hb, PCV%, ESR and WBCs (Table 3).

Table 3: Effect of Different Concentration of *Nigella Sativa L.* Seed Extract on Blood parameter (Hb, PCV, ESR, WBCs) of Adult Male Rats after Treated for 30 Days

Parameters Extract conc. ml/gm b.w	Hb g/dL	PCV %	WBCs c/cm
0	a 12.5	a 39.5	a 6500
0.1	b 13	a 40	b 7100
0.2	b 13.5	a 40.5	a 6450
0.3	b 13	b 41.5	a 6400
0.4	b 13.25	b 41.5	a 6400
L.S.D	0.233	1.04	151.6

L.S.D: Less Significant differences under ($p < 0.05$) for Hb=0.233, PCV=1.04 and W.B.Cs=151.60,

Different symbols mean significant effect.

Activities of GOT and GPT: The result obtained revealed that there were a significant changes ($p < 0.05$) in GOT and GPT

Table 4: Effect of Different Concentration of *Nigella sativa L.* Seed Extract on GOT and GPT in Serum of Adult Male Rats after Treated for 30 Days

Groups Parameters	Control group	0.1 ml/gm b.w.	0.2 ml/gm b.w.	0.3 ml/gm b.w.	0.4 ml/gm b.w.	Significant Level
GOT(U/L)(S.A.S.T)	a 3	b 2.71	b 4.1	a 3.15	b 3.7	
GPT (U/L) (S.A.L.T)	a 7.163	b 7.85	b 9.25	b 9.3	b 9.7	

L.S.D. Less Significant differences under under ($p \leq 0.05$) =0.171 Different symbols mean significant effect.

Histological Studies

Liver Tissue

Examination of H&E sections of livers of control shown that, the parenchyma was formed of classic hepatic lobules having the central veins in their middle, branching and anastomosing cords of hepatocytes radiate, and normal hepatocytes architecture and blood vessel (Figure 1a), while the group of normal rat administrated *N. sativa seed* extract showed vascular congestion of liver in 0.1 and 0.2 ml/gm b.w. (Figure 1b).

Spleen Tissue

Examination of H&E sections of spleen of control showed normal histological pictures (Figure 2a). Light microscopic observation of normal rat administered *N. sativa seed* extract, spleen showed hyperplasia in red pulps in all

doses of plant extract (Figure 2b).

Kidney Tissue

According to histopathological findings, kidney section of control and normal rat administered the *N. sativa seed* extract showed normal intact histological structure (Figure 3).

Pancreas Tissue

From the cross-section of the pancreas, the tissue structure of control and normal rat administered the *N. sativa seed* extract showed normal intact histological structure when examined by Light microscopic (Figure 4).

DISCUSSIONS

Herbal medicines have received great interest as an alternative to clinical therapy, and the demand for these therapies has currently increased rapidly. The increase in the number of users as opposed to the scarcity of scientific evidences on the safety of medicinal plants, have raised concerns regarding the toxicity and detrimental effects of these remedies (15), the current study was undertaken to evaluate and focus on the effect of *N. sativa* on some parameter of an animal model.

In present study the reduction in body weight may be due to the bitter tastes of the extract which may be significantly ($p < 0.05$) reduce the intake of food in compared with control group. The loss of appetite is often synonymous with weight loss due to disturbances in the metabolism of carbohydrate, protein, fat or other mechanism (16).

The presence of saponins in high concentrations has earlier been reported to induce weight loss in animal exposed to such plants due to its appetite-inhibiting effect. Thus, the presence of this phytochemical in *N. sativa* could account for the weight losing effect. (17). Thus, the presence of this phytochemical in *N. sativa* could account for the weight losing effect of *N. sativa*. In respect of relative organ weight, *N. sativa* produced variable effect on the selected organs. In the male rats, *N. sativa* produced increases in the relative pancreas weight of all dose, in the relative liver and spleen weight of the male rat where *N. sativa* produced significant reduction, and the relative kidney weight had no significant change.

In respect of relative organ weight, *N. sativa* produced variable effect on the selected organs. In the male rats, *N. sativa* produced increases in the relative pancreas weight of all the selected organs, in the relative liver and spleen weight of the female rat where *N. sativa* produced significant reduction, and the relative kidney weight had no significant change.

Similar results have been observed with *Cissus sicyoides* extract (18). Which reduced significantly body weight of rats, also (19). obtained that the aqueous extract of *Cissus sicyoides* decreased significantly body weight of rats when treated with 100,200 mg /kg of plant extract.

The body weight changes serve as a sensitive indication of the general health status of animals (20). The *N. sativa* extract interfere with the normal metabolism of animals as corroborated by the non significant difference from animals in the vehicle control group.

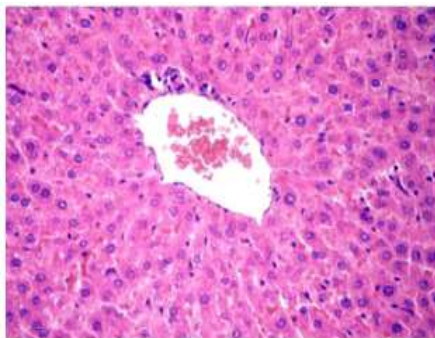


Figure 1a: A Photomicrograph in a Section of Control Rat Liver Showing the Central Vein Normal Hepatocytes Architecture. (HX & E. 400X)

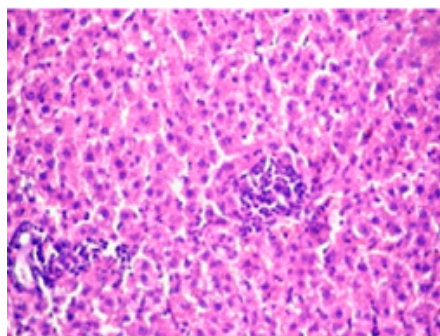


Figure 1b: Photomicrograph of Liver Section of Rats Treated with 0.1, 0.2, 0.3, 0.4 mg/gm of b.w. Showing Vacuolar Degeneration (HX & E. 400X)

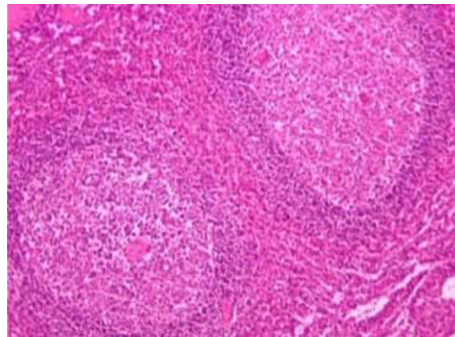


Figure 2a: A Photomicrograph in a Section of Control Rat Spleen Showing the Normal Architecture. (HX & E. 400X). RP: Red Pulp WP: WHITE Pulp CA: Central Artery. Spleen Tissue: 40X

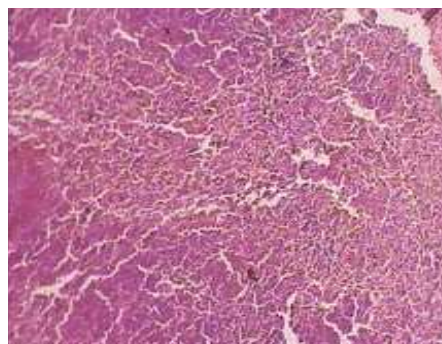


Figure 2b: A Photomicrograph in a Section Treated Rat Spleen Showing the Red Pulp Hyperplasia. (HX & E. 400X).100X Control

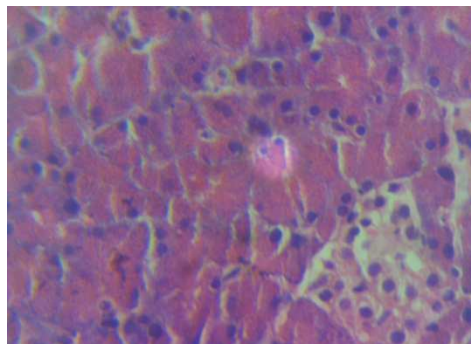


Figure 3: A Photomicrograph in a Section of Control Rat Pancreas Showing (1-Interlobular Septa, 2-Exocrine Gland, 3-Endocrine Iland)

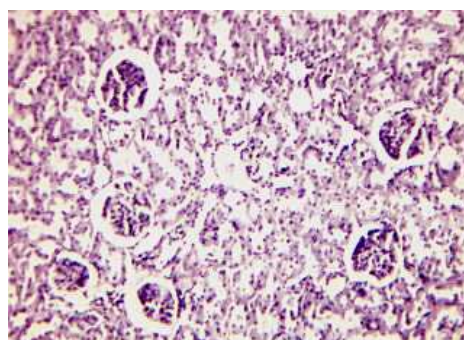


Figure 4: A Photomicrograph of a Section in the Kidney of the Control Rat Showing Normal Structure: Glomerulus's (G), and Renal Tubules (T). (HX & E. X400).

Similarly, no significant changes in the weights of the kidney was observed, suggesting that administration of *N. sativa* extract at all doses had no effect on the normal growth and it correlates with non histopathological changes(21). The results of our study revealed that the essential organs, such as liver and spleen were adversely affected throughout the treatment. Since there was reduction in body and relative organ weights of the treated animals at certain doses tested.

Blood parameters is an important index of physiological and pathological status in man and animals. Furthermore, such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies (22). In the present study, treatment for 30 days produced significant elevations in the measured haematological parameters indicating the haematopoietic effect. Thus, the significant elevations in PCV in concentrations 0.3 and 0.4 mg/gm of b.w. and Hb in concentrations 0.2, 0.3 and 0.4 mg/gm of b.w. strongly suggest that *N. sativa* could be useful in the management of anaemia. However, the significant thrombocytosis produced by *N. sativa* treatment suggests that while its haemopoietic effect could be beneficial in the management of anaemia, the resultant thrombocytosis could invoke predisposition to thrombotic stroke and ischaemic heart disease.(23)

The non significant effect of seed extract on total counts of WBCs due to non immune response of the rats to the extract. The neutrophils are the first line of defense in any microbial infection and are often significantly elevated in acute inflammatory conditions (24). while lymphocytes on the other hand produce antibodies that bind to pathogens to enable their destruction and are more involved in defense against intracellular microbes and tumour cells (25). This mean that the *NS* which stimulates the maturation and development of RBCs which in turn increases the level of Hb and PCV (26).

Liver function analysis is very important in the toxicity evaluation of drugs and plant extracts as it was necessary for the survival of an organism (27). GPT is the enzyme produced within the cells of the liver, recording increases in

conditions where liver cells have been inflamed or undergone cell death (28),(29). As the cells are damaged, the GPT leaks into the bloodstream leading to a rise in its serum concentrations (30). However, of these hepatic enzymes, GPT is the most sensitive and reliable marker of hepatocellular injury since GOT is known to be present in abundance in the cardiac muscles, skeletal muscles, kidneys and testes, and GPT abundant in the growing bone (31).

High levels of GPT and GOT are reported in liver diseases or hepatotoxicity (32). The non significant changes in GPT and GOT in male rats at all doses suggest that administration of *N. sativa* extract does not affect the hepatocyte function in the rats.

As a result, any disease state affecting any of these extrahepatic tissues significantly elevates the serum levels of these enzymes (31). From the results of oral administration of *N. sativa*, serum analyses of the treated rats showed that *N. sativa* treatment caused no significant alterations in the serum GPT and GOT levels indicating that *N. sativa* has no deleterious effect on liver functions. This is a strong indication of the oral safety of *N. sativa* on liver function.

Histopathological investigation of spleen did not exhibit any abnormalities in rats treated with low or high doses of NS implying no effect of this plant on hematopoiesis, and immunologic functions.

The macroscopic examinations of the organs of rats treated with various doses of *N. sativa* extract did not show any changes in color compared with control group rats' organs. Hypertrophy of organs is first hand indication of toxicity of chemical or biological substance. However, no hypertrophy of organs was observed in this study amongst all the groups studied. In addition, the microscopic examination revealed that none of the organs from the extract treated rats showed any alteration in cell structure or any unfavorable effects when viewed under the light microscope using multiple magnification powers. No pathologies were recorded in the histological sections of the vital organs (liver, spleen, kidney, and pancreas) of the control group. Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood (33). Thus, the non-significant increases observed in GPT and GOT strongly suggest that the administration of *N. sativa* extract did not alter the hepatocytes and, consequently, the metabolism of the rats as observed in the histopathology observations of liver tissue.

CONCLUSIONS

In light of these findings, we may conclude that *N. sativa* oil extract is not toxic in all the doses studied here. *N. sativa* oil extract did not cause any lethality or produce any remarkable histopathological signs. Further studies to determine the effects of *N. sativa* oil extract on an animal foetus, on pregnant animals, and their reproductive capacity are needed to complete the safety profile of this herb.

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